DEVELOPMENT OF A CHICK ASSAY FOR DETERMINING AVAILABILITY OF PHOSPHORUS FROM VARIOUS PHOSPHATE MATERIALS

By

BOBBY LEON DAMRON

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1968

ACKNOWLEDGEMENTS

The author is grateful to Dr. Robert H. Harms, Chairman of the Supervisory Committee, for his constant guidance, patience, and assistance in the planning and execution of the research reported in this dissertation. Appreciation is also expressed to Dr. J. L. Fry, Dr. H. R. Wilson, Dr. S. E. Grigsby, and Dr. C. B. Ammerman for their valuable suggestions and encouragement.

The author is indebted to Miss Kathleen Wall for her technical assistance, and to the undergraduate students for their assistance.

The cooperation of the Smith-Douglass Company, Norfolk, Virginia, is gratefully acknowledged for technical and financial contributions.

TABLE OF CONTENTS

TABLE OF CONTENTS--*Continued*

LIST OF TABLES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The element phosphorus was first prepared in the free state by Brandt, a German chemist, in 1669 and first recognized as an essential bone component by Gahn, a Swedish chemist, in 1769. Phosphorus probably plays a more varied role in the chemistry of living organisms than any other single element. It is also an essential constituent of proteins and fats occurring in muscular tissues, vital organs and brain. Phosphates are also known to be important buffers in tissue fluids.

Deficiencies of mineral elements in animal rations began to be recognized about 100 years ago when weak bones in cattle grazing in certain localities began to be associated with mineral inadequacies of the soil. According to Ewing (1963) the earliest recorded use of a phosphate feed supplement for the specific purpose of preventing a phosphorus deficiency in ruminants occurred in 1861 when Von Gohren reported that the weak bones of cattle grazing near the Rhine River could be prevented and cured by feeding small amounts of bone meal. Analysis of the soil and grass in these areas revealed a very low phosphorus and calcium content.

Weak bones in non-ruminants, particularly in swine, was noted as a serious problem around 1885, and it was found that feeding bone meal to pigs as a supplement to corn more than doubled the breaking strength of deficient bones.

Rock phosphate is the most abundant source of phosphorus, with approximately half of the 26 billion ton world reserve located in the United States. These phosphates were used as sources of phosphorus in plant nutrition as early as 1860. The belief that only phosphorus from plant and animal sources was available to animals accounted for the delay in its use in animal nutrition, and as late as 1914 it was still felt that at least a part of the animal's phosphorus requirement should be supplied in organic form.

The danger of fluorine toxicity began to be recognized as a serious problem around 1930, and, as a consequence, raw rock phosphates have now been largely replaced by sources that have been treated for the removal of fluorine. Defluorinated super phosphate, which was one of the first of these sources to be produced in commercial quantities, was in general use about 1940.

Considerable research has been conducted in recent years to determine which phosphorus supplements are suitable for use in poultry feeds. Among the sources recently

evaluated was colloidal phosphate, which is also called soft phosphate. Soft phosphate is obtained as a by-product during the mining of rock phosphate, and is an inexpensive source of supplemental phosphorus. The use of such a supplement in poultry diets would be of great importance from a commercial point of view.

The latest addition to the list of commercial phosphate feed supplements is a dicalcium phosphate which is made by treating phosphoric acid with the proper amount of lime. It is practically free of fluorine and contains up to 22 percent phosphorus as compared to approximately 15 percent in the best grades of bone meal and about 8.5 to 14.5 percent in the original defluorinated rock phosphates.

The National Research Council (1966), in a recent revision of its suggested nutrient requirements for chickens, increased the total dietary phosphorus requirement for starting chickens from 0.60 percent to 0.70 percent of the diet. Numerous studies have been conducted to establish this requirement for the chick, and there has been variation in reported requirements. McGinnis *et al.* (1944) reported that levels of greater than 0.58 percent phosphorus were required for maximum calcification. The available phosphorus requirement necessary for satisfactory

calcification was found by Singsen *et al.* (1947) to be between 0.38 and 0.47 percent of the diet. Gillis *et al.* (1949) and O'Rourke *et al.* (1952) found a requirement of approximately 0.50 percent. Grau and Zweigart (1953) indicated that maximum tibia ash of chicks was obtained with a level of not more than 0.45 percent phosphorus. Maximum calcification of chick bones was produced by a level of 0.58 percent total phosphorus in studies conducted by Fisher *et al.* (1953). A level of 0.76 to 0.81 percent phosphorus was suggested by Couch *et al.* (1937) as being adequate for normal growth and bone calcification of chicks up to 12 weeks of age. Additional studies were reviewed by Singsen *et al.* (1948), Gillis *et al.* (1949), and O'Rourke *et al.* (1952), and Nelson and Walker (1964).

Much of this variability in reported phosphorus requirements can probably be attributed to differences in availability of the phosphorus to the chick. The National Research Council (1966) states that at least 0.50 percent of the total feed of starting chickens should be inorganic phosphorus. All the phosphorus of non-plant feed ingredients is considered to be inorganic in nature. Approximately 30 percent of the phosphorus of plant products is non-Phytin phosphorus and may be considered as part of the inorganic phosphorus requirement. A portion of the phosphorus

requirement of growing chickens and laying and breeding hens must also be supplied in inorganic form, but this requirement for inorganic phosphorus is lower and not as well defined as it is for starting chickens. The interaction of vitamin D_3 has long been recognized as one of the factors governing the utilization of calcium and phosphorus from mineral sources. It has been demonstrated under certain conditions that levels of vitamin D_3 considerably in excess of those suggested by the National Research Council (1966) may improve the utilization of the phosphorus from certain low grade phosphate sources (Motzok *et al.*, 1965; Fritz and Roberts, 1966; and McKnight and Watts, 1966).

The advent of the computer has afforded the researcher and nutritionist with a valuable tool for the formulation of least-cost feeds. In return, the machine has increased the burden of its operator by demanding exacting specifications and analytical values for feeds and feedstuffs in order to function properly. In order for the nutritionist to formulate diets in the most economical and profitable manner he must have an accurate evaluation of the phosphorus content of these supplements and its availability. In the usual assay of phosphate supplements, the test sources are added to a phosphorus-deficient diet to supply graded sub-optimum levels

of phosphorus. The response, generally expressed in terms of bone calcification or body weight, is then compared to that from a standard source fed at equivalent total dietary phosphorus levels to establish a relative biological value for the test phosphate.

The interaction of calcium and phosphorus has long been recognized. Adverse Ca:P ratios limit the utilization of phosphorus, especially at sub-optimal levels such as are used in phosphorus assay diets. However, there is no standard calcium level or Ca:P ratio in general use. Nelson and Peeler (1964) indicated that one of the problems involved in the development of a biological assay for feed phosphates was whether to hold the calcium level constant or have a constant Ca:P ratio in the assay diet. In certain assays, constant calcium to phosphorus ratios were employed (Matterson *et al.*, 1945; Singsen and Scott, 1946; Creech *et al.*, 1956; Nelson and Peeler, 1961), while in others, constant calcium levels of 1.0 percent (Ammerman *et al.*, 1960) or 1.2 percent (Gardiner *et al.*, 1959) were used.

Recent studies by Waldroup *et al.* (1965a) pointed out that variation in the calcium content of the diet used in assaying phosphorus sources may influence significantly the relative biological value of a particular phosphate

source. In this report it was suggested that the use of a specific calcium level at different phosphorus levels appears to be desirable in order to elicit maximum response of the chick and allow full utilization of a particular phosphorus source. It is reasonable to assume that the most valid biological value would be obtained if the chick was able to maximally utilize the phosphorus present in the test diet. Therefore, a calcium level should be used which produces maximum growth and bone ash.

This series of experiments was designed to develop a standard assay curve for each of the commercial phosphate sources used in poultry diets. Such a standard curve should be of great value in eventually establishing a standard phosphorus assay that will give more realistic values of utilization, especially for test materials.

CHAPTER 2

DEVELOPMENT OF A CALCIUM STANDARD CURVE FOR MONOSODIUM PHOSPHATE

Monosodium phosphate has for many years been recognized and utilized as a standard phosphorus source in the determination of biological values and phosphorus requirements for poultry. These experiments were conducted to develop a standard calcium curve for this phosphorus reference material.

Experiment 1

Experimental Procedure

The basal diet used for this study was composed primarily of degerminated corn and soybean meal (Table 1). It was calculated to contain 22 percent protein and 2200 Calories of productive energy per kilogram of diet. Twenty-two hundred International Chick Units of supplemental vitamin D_3 were provided per kilogram of diet in order to eliminate any effect of this vitamin upon utilization. This diet was found, by analysis, to contain 0.30 percent total phosphorus and 0.26 percent calcium. This is a considerably higher calcium level than previously reported by Waldroup *et al.* (1965a) for this diet. The reason for this

TABLE 1

Composition of basal diet

Ingredient	Percent of diet
Degerminated corn meal	51.70
Cerelose	5.40
Soybean meal (50% protein)	34.00
Alfalfa meal (20% protein)	3.00
Iodized salt	0.40
Micro-ingredients[1]	0.50
Variable[2]	5.00
Percent protein	22.00
Productive energy (Cal./kg.)	2200
Percent phosphorus	0.30
Percent calcium	0.26

[1]Supplied per kilogram of diet: vitamin A, 6600 I.U.; vitamin D_3, 2200 I.C.U.; vitamin K, 2.2 mg.; riboflavin, 4.4 mg.; pantothenic acid, 13.2 mg.; niacin, 39.6 mg.; choline, 499.4 mg.; vitamin B_{12}, 22 mcg.; Santoquin, 0.0125%; manganese, 71.4 mg.; iron, 19.8 mg.; copper, 1.98 mg.; cobalt, 198 mg., iodine, 1.1 mg.; and zinc, 99 mcg.

[2]Calcium and phosphorus levels were obtained by altering the levels of reagent grade calcium carbonate, phosphate source, and yellow builders sand.

difference was not determined; however, it points out the necessity of chemical analysis of the basal diet used with each set of ingredients. Reagent grade monosodium phosphate ($NaH_2PO_4 \cdot H_2O$) was used to supply supplemental phosphorus in 0.05 percent increments to a total of 0.55 percent total phosphorus.

Within each phosphorus level, reagent grade calcium carbonate was used to supply supplemental calcium. Five calcium levels were used at each phosphorus level. Levels were selected which were estimated from previous studies (Waldroup *et al.*, 1965a) to result in a response line and plateau within each phosphorus level so that the appropriate calcium requirement for that particular phosphorus level could be selected.

Three successive trials were conducted. In each trial, three replicate groups of five male and five female broiler-type chicks (Vantress × White Plymouth Rock) per group were fed each diet from 1 to 21 days of age. The chicks were sexed, debeaked, and randomly assigned to treatment groups at one day of age.

The chicks were brooded in Oakes 801-A five deck thermostatically controlled, electrically heated, battery brooders with raised wire floors. At the termination of each trial, all birds were individually weighed, and two males and two females from each group were sacrificed for

tibia ash determinations. The vitamin D determination procedure of the Association of Official Agricultural Chemists (1965) was followed in ashing the bones. Statistical analysis (Analysis of Variance; Snedecor, 1956) of the data failed to reveal a treatment × trial interaction; therefore, the data from the three trials were combined. Significant differences between tibia ash and body weight treatment means were determined by Duncan's multiple range test (1955). Based on these measurements, the ideal calcium level was selected for each level of phosphorus, and a standard curve of calcium levels determined for the various additions of monosodium phosphate.

Results and Discussion

The level of supplemental calcium fed with each level of phosphorus significantly influenced tibia ash and body weight (Table 2). However, the greatest influence was noted with tibia ash since responses were obtained with higher levels of calcium, especially at the higher phosphorus levels. The ideal calcium level was different for the five different phosphorus levels.

Maximum tibia ash and body weight were obtained with 0.18 percent supplemental calcium when the diet contained 0.30 percent phosphorus (Table 2). However,

TABLE 2

Tibia ash and body weight of chicks fed various levels of phosphorus and calcium supplied from monosodium phosphate and reagent grade calcium carbonate

Total phosphorus[1] (%)	Calcium[1] (%)		Tibia ash[2] (%)	Body weight[2] (grams)
	Supplemental	Total		
0.30	0.03	0.29	28.6a	210a
	0.08	0.34	32.1de	250bcd
	0.13	0.39	33.6fgh	265cde
	0.18	0.44	35.3ij	271ef
	0.23	0.49	33.7fgh	280efg
0.35	0.03	0.29	29.8bc	242cd
	0.09	0.35	32.6efg	267de
	0.15	0.41	36.7jk	293g
	0.21	0.47	36.9kl	298gh
	0.27	0.53	36.5jk	290g
0.40	0.03	0.29	33.0efg	240bc
	0.10	0.36	34.3ghi	280efg
	0.17	0.43	37.3kl	302h
	0.24	0.50	38.7lm	299gh
	0.31	0.57	39.2mn	303h
0.45	0.03	0.29	29.2ab	225ab
	0.11	0.37	33.4efgh	281efg
	0.19	0.45	39.9no	302h
	0.27	0.53	38.5lm	310h
	0.35	0.61	39.1mm	313h
0.50	0.03	0.29	30.8cd	237ab
	0.12	0.38	35.3ij	291g
	0.21	0.47	38.3lm	314h
	0.30	0.56	40.1no	316h
	0.39	0.65	40.2no	309h
0.55	0.03	0.29	31.3d	220a
	0.13	0.39	34.5hi	284fg
	0.23	0.49	39.6mm	297gh
	0.33	0.59	41.2o	361h
	0.43	0.69	41.4o	309h

[1]The basal diet contained 0.30 percent phosphorus and 0.26 percent calcium.

[2]Means with different letters are significantly different according to Duncan's multiple range test.

body weight was not different with levels of 0.13 or 0.23 percent supplemental calcium. Levels of 0.15, 0.21, and 0.27 percent supplemental calcium with 0.35 percent phosphorus resulted in maximum tibia ash and body weights (Table 2). Supplemental calcium levels of less than 0.15 percent resulted in a significant lowering of the tibia ash and body weight.

Maximum growth was obtained with supplemental calcium levels of 0.17, 0.24, and 0.31 percent, when the diet contained 0.40 percent phosphorus (Table 2). However, a level of 0.17 percent supplemental calcium did not support maximum tibia ash. This would indicate a level of 0.24 percent supplemental calcium as being ideal for this level of phosphorus.

Levels of 0.19 and 0.35 percent supplemental calcium supported both maximum tibia ash and body weight (Table 2) when the diet contained 0.45 percent phosphorus. The level of 0.27 percent supplemental calcium supported maximum body weight; however, tibia ash was significantly lower than for the group receiving 0.19 percent supplemental calcium. The lowered tibia ash was attributed to chance since the tibia ash was also numerically greater for groups receiving 0.35 percent supplemental calcium.

When the diet contained 0.50 percent phosphorus, levels of 0.21, 0.30, and 0.39 percent supplemental calcium

supported maximum body weight (Table 2). However, tibia ash was significantly lower when the diet contained only 0.21 percent supplemental calcium.

Maximum body weight was obtained with supplemental calcium levels of 0.23, 0.33, and 0.43 percent when the diet contained 0.55 percent phosphorus (Table 2). However, a significantly lower tibia ash was obtained with the diet containing 0.23 percent calcium.

Based on the above results, a standard curve has been suggested for use in selection of the proper level of calcium to use in order to elicit maximum response, measured as both tibia ash and body weight. The suggested calcium levels are 0.44, 0.47, 0.50, 0.53, 0.56, and 0.59 percent for phosphorus levels of 0.30, 0.35, 0.40, 0.45, 0.50, and 0.55 percent, respectively, when supplemental phosphorus is supplied as monosodium phosphate ($NaH_2PO_4 \cdot H_2O$). It should be emphasized, however, that these levels are only for the basal diet employed in this experiment and may not apply when dietary modifications are made.

Increasing the phosphorus:calcium ratio by the addition of phosphorus to diets containing calcium levels of 0.29, 0.39, 0.49, and 0.53 percent did not depress performance of chicks when measured as tibia ash or growth rate (Table 2). An improvement of tibia ash was produced

by widening the P:Ca ratio with the addition of phosphorus to the diet containing the low level of calcium (0.29 percent total calcium). This was in contrast to the effect of widening the calcium:phosphorus ratios at low levels of phosphorus as reported by Vandepopuliere *et al.* (1961).

Experiment 2

Experimental Procedure

A second experiment was conducted to determine whether the suggested calcium levels would produce maximum tibia ash and body weight when phosphorus was fed at other levels. The same general procedure was followed as used in experiment 1 except that four, instead of three, replicates of five males and five females were used per treatment. Also, tibia ash was determined on five males per treatment instead of two males and two females per treatment as in experiment 1. Levels of 0.07, 0.14, and 0.21 percent phosphorus from monosodium phosphate ($NaH_2PO_4 \cdot H_2O$) were added to the basal diet and five suitable levels of calcium were fed at each of the three levels of phosphorus.

Results and Discussion

A level of 0.19 percent supplemental (0.45 percent total) calcium gave maximum tibia ash and body weight when

0.07 percent supplemental phosphorus was added (Table 3). This is in fair agreement with the 0.485 percent suggested on the standard curve (experiment 1), and a level of 0.49 percent calcium did not significantly reduce either body weight or tibia ash.

When 0.14 percent phosphorus was added, a level of 0.26 percent supplemental calcium produced maximum tibia ash and body weight. However, when the diet contained 0.21 percent supplemental phosphorus, a level of 0.30 percent supplemental calcium gave maximum performance. These levels agree very well with those suggested on the standard curve. These data indicated that the suggested levels of calcium (experiment 1) would elicit maximum tibia ash and body weight. Therefore, it is suggested that such a curve be utilized in phosphorus assays for determining the level of calcium to use with this diet.

Based on the data in experiment 2 (Table 3), it would appear that these levels have a sufficient margin of safety for normal variation in calcium content of the basal diet. Although the chicks grew at a higher rate in this experiment, the response to the various dietary phosphorus and calcium levels was essentially the same as observed in the first experiment.

TABLE 3

Tibia ash and body weight of chicks fed various levels of phosphorus and calcium supplied from monosodium phosphate and reagent grade calcium carbonate

Total phosphorus[1] (%)	Calcium[1] (%) Supplemental	Calcium[1] (%) Total	Tibia ash[2] (%)	Body weight[2] (grams)
	0.13	0.39	36.1f	344cd
	0.16	0.42	35.8f	347bcd
0.37	0.19	0.45	36.1f	339de
	0.23	0.49	34.6f	325de
	0.27	0.53	34.8f	313e
	0.19	0.45	39.9cde	364abc
	0.22	0.48	39.7cde	375a
0.44	0.26	0.52	40.2bcd	369abc
	0.30	0.56	39.1de	350bcd
	0.34	0.64	38.2e	311e
	0.23	0.49	41.2abc	372ab
	0.26	0.52	41.2abc	382a
0.51	0.30	0.56	42.4a	381a
	0.34	0.60	41.4abc	372ab
	0.42	0.68	41.7ab	345cd

[1]The basal diet contained 0.30 percent phosphorus and 0.26 percent calcium.

[2]Means with different letters are significantly different according to Duncan's multiple range test.

Summary

An experiment, involving three trials, was conducted using reagent grade monosodium phosphate and calcium carbonate to establish a calcium standard curve for use in phosphorus availability studies. Six levels of supplemental phosphorus (0 to 0.25 percent) were added to the degerminated corn-soybean meal basal diet, with five calcium levels per phosphorus level.

Broiler-type chicks were fed the experimental diets for 21 days, and tibia ash data and body weights were used to determine the calcium requirement at each phosphorus level. These data were used to construct a curve giving the suggested calcium level for any level of monosodium phosphate when using a diet based largely upon degerminated corn and soybean meal.

Results from a second experiment indicated that calcium levels selected from the standard curve for other levels of monosodium phosphate would produce maximum bone ash and body weight.

CHAPTER 3

DEVELOPMENT OF A CALCIUM STANDARD CURVE FOR DEFLUORINATED PHOSPHATE

Defluorinated phosphate is one of the most widely used sources of phosphorus in poultry diets because of the abundance of raw material, its low fluorine content, and the fact that it has a high concentration of readily available calcium and phosphorus. The Ca:P ratio of defluorinated phosphate is quite similar to that found in bone, and this is considered to be another advantage for this material.

Gillis *et al.* (1954), using biological assay techniques and beta tricalcium phosphate as a standard, found defluorinated phosphate to have a biological value varying from 82 to 99 percent, depending upon the method of manufacture.

The experiment reported herein was conducted to develop a standard calcium curve which will allow researchers to more accurately evaluate the biological availability of this feed grade phosphorus supplement.

Experimental Procedure

Two successive trials were conducted. Three replicate groups, each containing five male and five female

broiler-type chicks (Vantress × White Plymouth Rock), received each dietary treatment. The chicks were sexed, debeaked, and randomly assigned to the treatments at one day of age. The birds received the experimental diets and tap water *ad libitum* from 1 to 21 days of age.

The basal diet used for this study was identical to the one described in Chapter 2 (Table 1). Commercial grade defluorinated phosphate was used to supply supplemental phosphorus in 0.07 percent increments to a total dietary level of 0.51 percent phosphorus (Table 4).

Within each phosphorus level, reagent grade calcium carbonate was used to supply supplemental calcium. Five equally spaced calcium levels were used at each phosphorus level in an attempt to select the appropriate calcium level which would give a maximum response with each particular phosphorus level.

The battery brooders used in this experiment were the same as those described in Chapter 2. At the termination of each trial the birds were separated and weighed according to sex. Two males and two females from each group were sacrificed for tibia ash determinations. The vitamin D determination procedure of the Association of Official Agricultural Chemists (1965) was followed in ashing the bones. Statistical analysis of the data

(Analysis of Variance; Snedecor, 1956) failed to reveal a treatment × trial interaction; therefore, the data from the two trials were combined.

Significant differences between tibia ash and body weight treatment means were determined by Duncan's multiple range test (1955). Based on these measurements, the ideal calcium level was selected for each level of phosphorus, and a standard curve of calcium levels determined for the various additions of defluorinated phosphate.

Results and Discussion

The combined results of the two trials (Table 4) indicate that the level of supplemental calcium was more critical at the lowest level of phosphorus supplementation. There were no significant differences between tibia ash values within a given level of phosphorus; however, each increment of phosphorus resulted in a significant increase of tibia ash values. Body weight also tended to increase numerically as the level of phosphorus increased; however, trends of statistical significance were not as clear cut.

When the diet contained 0.37 percent phosphorus, the highest levels of bone ash resulted from the feeding of 0.475 and 0.520 percent total calcium. Since these two treatments also produced the heaviest body weights of

TABLE 4

Tibia ash and body weight of chicks fed various levels of phosphorus and calcium supplied from defluorinated phosphate and reagent grade calcium carbonate

Total phosphorus[1] (%)	Calcium[1] (%) Supplemental	Total	Tibia ash[2] (%)	Body weight[2] (grams)
	. . .	0.385	33.7a	295a
	0.045	0.430	33.9a	293a
0.37	0.090	0.475	34.4a	313bc
	0.135	0.520	34.8a	310b
	0.180	0.565	34.1a	305ab
	. . .	0.510	37.3b	333de
	0.050	0.560	38.7b	337de
0.44	0.100	0.610	37.5b	327cd
	0.150	0.660	38.3b	331de
	0.200	0.710	38.1b	332de
	. . .	0.635	40.9c	347e
	0.025	0.660	40.3c	337de
0.51	0.050	0.685	41.1c	347e
	0.075	0.710	40.6c	344e
	0.100	0.735	41.1c	346e

[1]The basal diet contained 0.30 percent phosphorus and 0.26 percent calcium.

[2]Means with different letters are significantly different according to Duncan's multiple range test.

any diet containing 0.37 percent phosphorus, an intermediate calcium level of 0.498 percent was selected as one providing an adequate safety margin for this level of phosphorus.

Changing the calcium level when the diet contained 0.44 percent phosphorus did not produce a significant increase of body weight or bone ash, but the group that received 0.560 percent total dietary calcium had numerically superior body weight and bone ash. In order to allow for a margin of safety around the value selected for the curve, 0.590 percent total calcium was selected for this level of phosphorus.

When the diet contained 0.51 percent phosphorus, a level of 0.685 percent calcium resulted in a combination of the highest body weight and greatest percent bone ash of any of the calcium levels tested. Since there were no significant differences between either the body weights or bone ash of this series and very little fluctuation among numerical values, it was felt that the choice of 0.685 percent total calcium would afford an ample margin of safety on either side of the standard curve.

Summary

An experiment consisting of two trials was conducted to establish a calcium standard curve for use in phosphorus availability studies involving defluorinated phosphate.

Three levels of supplemental phosphorus (0.07, 0.14, and 0.21 percent) supplied from defluorinated phosphate were added to the degerminated corn-soybean meal basal diet. Five equally spaced calcium levels were fed with each level of phosphorus.

Broiler-type chicks received the experimental diets from 1 to 21 days of age; tibia ash and body weight data were used as the criteria for determining the calcium requirement at each phosphorus level. The optimum calcium levels selected were 0.498, 0.590, and 0.685 percent, respectively, for total phosphorus levels of 0.37, 0.44, and 0.51 percent.

CHAPTER 4

DEVELOPMENT OF A CALCIUM STANDARD CURVE FOR DICALCIUM PHOSPHATE

Dicalcium phosphate is a highly available phosphate material that is widely utilized as a source of inorganic phosphorus for poultry diets. Gillis *et al.* (1954) and Nelson and Peeler (1961), using beta-tricalcium phosphate as the reference material, found dicalcium phosphate to have a biological value of 98 and 97 percent, respectively.

This experiment was conducted to determine the levels of calcium necessary for the maximum expression of biological availability by this commercial phosphate source at various levels of phosphorus supplementation.

Experimental Procedure

Three successive trials were conducted. In each trial three replicate groups containing five male and five female broiler-type chicks (Vantress × White Plymouth Rock) received each dietary treatment and tap water *ad libitum* from 1 to 21 days of age. The chicks were sexed, debeaked, and randomly assigned to treatment groups at one day of age.

The basal diet used was identical to the one described in Chapter 2 (Table 1). Commercial grade

dicalcium phosphate was used to supply supplemental phosphorus in 0.07 percent increments to a total dietary level of 0.51 percent (Table 5). Within each phosphorus level reagent grade calcium carbonate was used to provide five equally spaced supplemental calcium levels. This range of calcium supplementation was provided for each phosphorus level in order that the appropriate calcium requirement for each level might be determined.

The battery brooders used in this experiment were identical to the ones described in Chapter 2. At the termination of each trial the birds were separated and weighed according to sex, and two males and two females from each replicate group were sacrificed for tibia ash determinations. The vitamin D determination procedure of the Association of Official Agricultural Chemists (1965) was followed in ashing the bones. Statistical analysis of the data (Analysis of Variance; Snedecor, 1956) did not reveal a significant treatment × trial interaction; therefore, the data from the three trials were combined.

Significant differences between tibia ash and body weight treatment means were determined by Duncan's multiple range test (1955). Based on these measurements, the ideal calcium level for each level of phosphorus was selected, and a standard curve of calcium levels determined for the various additions of dicalcium phosphate.

Results and Discussion

The combined results of the three trials with dicalcium phosphate are presented in Table 5. A significant increase of tibia ash and body weight resulted when the total calcium level was increased from 0.35 to 0.43 percent in diets containing 0.37 percent total phosphorus. The diet containing 0.43 percent calcium produced the largest numerical bone ash value of any group receiving 0.37 percent phosphorus, and no further significant improvement of tibia ash or body weight resulted from feeding higher calcium levels with this level of phosphorus. In order that a margin of safety might be allowed around the calcium level proposed for this level of phosphorus, a total calcium level of 0.47 percent was selected.

The tibia ash of birds receiving diets containing 0.44 percent total phosphorus was significantly increased when the dietary calcium level was increased from 0.44 to 0.50 percent; however, there were no significant differences noted among body weights. The diet containing 0.44 percent phosphorus and 0.56 percent calcium resulted in tibia ash and body weight values which were numerically superior to any of the treatment groups receiving 0.44 percent phosphorus. However, it was considered that since consistent numerical improvements of tibia ash and body weight

TABLE 5

Tibia ash and body weight of chicks fed various levels of phosphorus and calcium supplied from dicalcium phosphate and reagent grade calcium carbonate

Total phosphorus[1] (%)	Calcium[1] (%)		Tibia ash[2] (%)	Body weight[2] (grams)
	Supplemental	Total		
	0.02	0.35	30.8a	315a
	0.10	0.43	36.2bc	346cd
0.37	0.18	0.51	35.1b	348cd
	0.26	0.59	35.2b	328ab
	0.34	0.67	35.8b	334bc
	0.04	0.44	35.9b	337bcd
	0.10	0.50	37.7cd	340bcd
0.44	0.16	0.56	38.7def	353d
	0.22	0.62	38.6def	336bcd
	0.28	0.68	38.3de	350cd
	0.07	0.53	39.9ef	346cd
	0.11	0.57	39.9ef	348cd
0.51	0.15	0.61	40.2f	346cd
	0.19	0.65	40.3f	344bcd
	0.23	0.69	39.7ef	349cd

[1]The basal diet contained 0.30 percent phosphorus and 0.26 percent calcium.

[2]Means with different letters are significantly different according to Duncan's multiple range test.

resulted from the feeding of 0.44, 0.50, and 0.56 percent calcium, a level somewhat in excess of 0.56 percent was required in order to afford some factor of safety. Since a dietary level of 0.62 percent calcium with 0.44 percent phosphorus produced a tibia ash value that was almost equal to that resulting from the feeding of 0.56 percent calcium, an intermediate total calcium level of 0.58 percent was selected as the second point of the standard curve for the assay of dicalcium phosphate.

Increasing the level of calcium with 0.51 percent phosphorus produced essentially no response within either criterion, as there were no significant differences (and only very small numerical variations) between any of the tibia ash or body weight values. These results were not unexpected since 0.51 percent phosphorus is approaching the bird's requirement, and it is well established that dietary calcium levels become less critical as the phosphorus content of the diet is increased. In order to provide an adequate safety margin, 0.69 percent total dietary calcium was selected for a level of 0.51 percent phosphorus.

Summary

This experiment, comprised of three trials, was conducted to define a calcium standard curve for use in studying the availability of phosphorus from commercial

grade dicalcium phosphate. Three levels of supplemental phosphorus (0.07, 0.14, and 0.21 percent) were provided from dicalcium phosphate, and five equally spaced levels of supplemental calcium supplied from reagent grade calcium carbonate were fed with each level of phosphorus in a degerminated corn-soybean meal basal diet.

Broiler-type chicks housed in starting batteries received the experimental diets from 1 to 21 days of age. Tibia ash and body weight data were used as the criteria for determining the optimum level of calcium to be fed with each level of phosphorus. The calcium levels selected were 0.47, 0.58, and 0.69 percent, respectively, for total phosphorus levels of 0.37, 0.44, and 0.51 percent.

CHAPTER 5

DEVELOPMENT OF A CALCIUM STANDARD CURVE FOR SOFT PHOSPHATE

In recent years there has been considerable interest in the utilization of soft phosphate, also known as colloidal phosphate or phosphatic clay, as a source of phosphorus in poultry diets. Soft phosphate is one of the materials with low phosphorus content (approximately 18 percent calcium and 9 percent phosphorus), yet it is also one of the most inexpensive sources of supplemental phosphorus.

The main problem associated with the use of this material is the low biological availability of its phosphorus and the wide range of reported availability values. Nelson and Peeler (1961) reported an availability of only 34 percent for the phosphorus of soft phosphate. In contrast, Waldroup *et al.* (1965a) estimated that 51-59 percent of the phosphorus in soft phosphate was available and suggested that the dietary calcium level could influence availability.

This experiment was conducted in order to determine the levels of dietary calcium necessary for the maximum expression of biological availability by this phosphate source.

Experimental Procedure

In each of three successive trials, three replicate groups containing five male and five female broiler-type chicks (Vantress × White Plymouth Rock) received each dietary treatment and tap water *ad libitum* from 1 to 21 days of age. The birds were sexed, debeaked, and randomly assigned to treatment groups at one day of age.

The basal diet used was identical to the one described in Chapter 2 (Table 1). Supplemental phosphorus supplied from commercial grade soft phosphate was added in 0.07 percent increments to result in total dietary phosphorus levels of 0.37, 0.44, and 0.51 percent. Within each phosphorus level, reagent grade calcium carbonate was used to supply five equally spaced supplemental calcium levels (Table 6). The levels of calcium supplementation varied among the three phosphorus levels and were provided in order that the appropriate calcium requirement for each level of phosphorus might be determined.

The battery brooders used in this experiment were identical to the ones described in Chapter 2. On the final day of each trial, the birds were separated and weighed according to sex, and two males and two females from each replicate group were sacrificed for tibia ash determinations. The vitamin D determination procedure of the

Association of Official Agricultural Chemists (1965) was followed in ashing the bones. Statistical evaluation of the data (Analysis of Variance; Snedecor, 1956) revealed no significant treatment × trial interaction; therefore, the data from the three trials were combined.

Significant differences between tibia ash and body weight treatment means were determined by Duncan's multiple range test (1955). Based on these measurements, the ideal calcium level for each phosphorus level was selected, and a standard curve of calcium levels determined for the various additions of soft phosphate.

Results and Discussion

The combined results of the three trials with soft phosphate are shown in Table 6. There were no significant differences among the body weights of birds receiving 0.37 percent total phosphorus, regardless of the calcium level fed. The addition of 0.06 and 0.12 percent supplemental calcium to a diet containing 0.07 percent phosphorus from soft phosphate (0.37 percent total phosphorus) resulted in a significant increase of bone ash for each of these calcium additions. No further significant improvements were noted by increasing the supplemental calcium level above 0.12 percent (0.52 percent total). The birds receiving a diet containing 0.12 percent supplemental

TABLE 6

Tibia ash and body weight of chicks fed various levels of phosphorus and calcium supplied from soft phosphate and reagent grade calcium carbonate

Total phosphorus[1] (%)	Calcium[1] (%)		Tibia ash[2] (%)	Body weight[2] (grams)
	Supplemental	Total		
0.37	. .	0.40	31.5a	311a
	0.06	0.46	34.4b	318a
	0.12	0.52	36.3cde	323ab
	0.18	0.58	35.6bcde	313a
	0.24	0.64	35.1bcd	326abc
0.44	. .	0.54	36.6de	338bcd
	0.04	0.58	37.0e	341cd
	0.08	0.62	36.3cde	352d
	0.12	0.66	36.7de	339bcd
	0.16	0.70	34.8bc	343d
0.51	. .	0.68	40.1f	355d
	0.02	0.70	39.6f	349d
	0.04	0.72	40.3f	347d
	0.06	0.74	39.5f	344d
	0.08	0.76	38.9f	347d

[1]The basal diet contained 0.30 percent phosphorus and 0.26 percent calcium.

[2]Means with different letters are significantly different according to Duncan's multiple range test.

calcium (0.52 percent total) had the greatest tibia ash value resulting from any of the diets containing 0.37 percent phosphorus. Since a margin of safety was needed on the total calcium level selected, it was proposed that a dietary calcium level of 0.56 percent would be appropriate for this phosphorus level.

Supplementing the diet containing 0.44 percent total phosphorus with graded levels of reagent grade calcium carbonate resulted in no significant improvement of either tibia ash or body weight. However, a level of 0.16 percent supplemental calcium (0.70 percent total) produced a statistically significant depression of tibia ash. The addition of 0.04 percent supplemental calcium (0.58 percent total) resulted in a tibia ash value which was numerically superior to that of any other calcium level. The diet containing 0.08 percent supplemental calcium (0.62 percent total) produced the best body weight of any diet containing 0.44 percent total phosphorus. A level of 0.62 percent total calcium was selected for feeding with 0.44 percent total phosphorus.

None of the calcium levels supplied with 0.51 percent total phosphorus provided a significantly different response as measured by tibia ash or body weight; however, there was a slight numerical decrease of tibia ash as the total calcium level was increased above 0.72 percent.

Again, this indicates that the level of calcium becomes less critical as the dietary phosphorus level approaches the bird's requirement. A level of 0.68 percent total calcium was selected for feeding with 0.51 percent total phosphorus because this dietary combination produced the greatest body weight of any diet containing 0.51 percent total phosphorus, and near maximum tibia ash.

Summary

An experiment consisting of three trials was conducted in order to define a calcium standard curve which would be of value in studying the availability of phosphorus from soft phosphate. Three levels of supplemental phosphorus (0.07, 0.14, and 0.21 percent) were provided from soft phosphate, and five equally spaced supplemental calcium levels supplied from reagent grade calcium carbonate were fed with each level of phosphorus in a degerminated corn-soybean meal basal diet. Both the supplemental and total calcium levels varied among the different levels of phosphorus.

Broiler-type chicks housed in starting batteries received the experimental diets from 1 to 21 days of age. Body weight and tibia ash data were used as the criteria for determining the optimum level of calcium to be fed with each level of phosphorus. With due consideration

having been given to safety margins, the total calcium levels selected were 0.56, 0.62, and 0.68 percent, respectively, for total phosphorus levels of 0.37, 0.44, and 0.51 percent.

CHAPTER 6

INFLUENCE OF DIET COMPOSITION ON THE UTILIZATION OF SOFT PHOSPHATE IN BROILER DIETS

Numerous studies have been conducted to evaluate the biological value of soft phosphate for poultry. The majority of these reports has indicated that the phosphorus from soft phosphate is poorly utilized. Nelson and Peeler (1961) reported a biological value of only 34 percent for soft phosphate and found that the availability of poor quality phosphates was not improved when fed in a mixture with materials possessing a higher biological availability.

In addition, Summers *et al.* (1959) reported the availability of phosphorus from soft phosphate to be approximately 47 percent, and Waldroup *et al.* (1965a) estimated that 51-59 percent of the phosphorus in soft phosphate was available.

Summers *et al.* (1959) found that the availability of soft phosphate was improved by the addition of either phosphoric or hydrochloric acid. In contrast, Waldroup *et al.* (1965c) reported no apparent improvement in the utilization of phosphorus from soft phosphate by combining it with phosphoric acid. These workers concluded that the

apparent increased phosphorus content of the mixture could be attributed to the loss of water vapor during the chemical reaction that occurred as the materials were combined.

Waldroup *et al.* (1963) found that when dietary calcium and phosphorus levels were suboptimal, the calcium:phosphorus ratio was more critical in diets containing soft phosphate than in those diets containing a more readily available phosphorus source. It has been demonstrated under certain conditions that levels of vitamin D_3 considerably in excess of those suggested by the National Research Council (1966) may improve the utilization of the phosphorus from soft phosphate (Motzok *et al.*, 1965; Fritz and Roberts, 1966; and McKnight and Watts, 1966).

The experiments reported in this chapter were conducted to study the influence of various dietary factors on the utilization of phosphorus from soft phosphate, and to attempt to obtain maximum growth with broiler-type chicks when soft phosphate was utilized as the sole supplemental source of phosphorus.

Experimental Procedure

Day-old broiler-type chicks, obtained from a commercial hatchery, were utilized in all experiments. Ten male and ten female chicks were randomly assigned to

floor pens according to a randomized block design. Four replicate pens were assigned to each dietary treatment. All pens contained 2.32 square meters of floor area, and were uniform in shape and construction. Each pen was provided with one automatic waterer, one hanging cylindrical feeder, and one infrared heat lamp. Dried peanut hulls were used as litter.

Treatments consisted of graded additions of soft phosphate to the basal diets shown in Table 7. In experiments 1 and 2, and one-half of experiment 3, a basal diet (Table 7, diet 1) containing 0.34 percent phosphorus and 0.16 percent calcium was used. The fish meal basal (Table 7, diet 2) of experiment 3 contained 0.46 percent phosphorus and 0.28 percent calcium. Since the vitamin D_3 level of feeds has been shown to influence calcium and phosphorus utilization, all experimental diets were fortified with 7920 I.C.U. of vitamin D_3/kg.

All diets were kept iso-caloric (2093 Cal./kg.) and iso-nitrogenous (22.05 percent protein) by varying the level of corn, soybean oil meal, and animal fat. Values of Maddy *et al.* (1963) were used in the calculation of adjustments. The birds were given the experimental diets and water *ad libitum* from one day of age until the end of the test.

TABLE 7

Composition of basal diets

Ingredients	1	2
	(Percent of diet)	
Yellow corn	47.41	51.60
Soybean meal (50%)	35.50	30.60
Fish meal (60%)	. . .	2.50
Alfalfa meal (20%)	3.00	3.00
Animal fat	5.74	3.30
Micro-ingredients[1]	0.50	0.50
Iodized salt	0.40	0.40
Variable ingredients[2]	7.45	8.10

[1]Micro-ingredient mix supplied per kilogram of diet: 6,600 I.U. vitamin A, 449.4 mg. choline, 39.6 mg. niacin, 7,920 I.C.U. vitamin D_3, 4.4 mg. riboflavin, 13.2 mg. pantothenic acid, 22 mcg. vitamin B_{12}, 0.0275 percent Santoquin, 19.8 mg. iron, 1.98 mg. copper, 198 mcg. cobalt, 11 mg. iodine, 99 mcg. zinc and 220 mg. of manganese sulfate.

[2]Variable ingredients included sources of supplemental phosphorus and calcium and an inert filler.

Body weight and tibia ash were used as criteria of evaluation. In addition, feed efficiency values were calculated for experiments 2 and 3. The birds from each pen were grouped by sex and weighed at four weeks of age in experiments 1 and 2, and at four and eight weeks of age in experiment 3. In experiments 2 and 3, two males and two females from one pen of each dietary treatment were sacrificed at four weeks of age for bone ash determinations. The left tibia was removed, cleaned of adhering tissue and individually ashed according to the method outlined by the Association of Official Agricultural Chemists (1965). Feed consumption was also determined at four weeks of age in experiment 2, and at four and eight weeks of age for experiment 3.

The data from these experiments were subjected to the analysis of variance as outlined by Snedecor (1956). Significant differences between treatment means were determined by the multiple range test of Duncan (1955).

Experiment 1

A 3 × 4 factorial arrangement of treatments was used involving three supplemental phosphorus levels (0.40, 0.50, and 0.60 percent) and four levels of supplemental calcium (0, 0.20, 0.30, and 0.40 percent) added to the basal diet (Table 7, diet 1). Two positive control

diets consisting of diet 1 supplemented to provide adequate levels of phosphorus (0.70 percent) and calcium (0.80 and 1.00 percent) from commercial grade dicalcium phosphate (21.69 percent phosphorus and 20.92 percent calcium) and ground limestone were also fed. The diets of this experiment were fed from one day until four weeks of age, at which time the test was terminated.

Experiment 2

This experiment was also of four weeks duration, starting when the chicks were housed at one day of age. The basal diet was supplemented with soft phosphate in order to provide levels of 0.40, 0.50, and 0.60 percent supplemental phosphorus. Ground limestone was added to supply supplemental calcium levels ranging from 0 to 0.40 percent (Table 9) to diets containing each of the supplemental phosphorus levels. Two positive control diets, identical to those used in experiment 1, were also fed.

Experiment 3

One-half of the treatments of this experiment were essentially replicates of a portion of experiments 1 and 2 in that similar additions of soft phosphate and ground limestone were made to the basal diet (Table 7, diet 1). Supplemental phosphorus levels of 0.40 and 0.50 percent and supplemental calcium levels as shown in Table 10 were

provided from these sources. Two positive control diets containing adequate levels of phosphorus (0.70 percent) and calcium (0.80 percent and 1.00 percent) supplied from dicalcium phosphate and ground limestone were also fed. These control diets were identical to the ones fed in experiments 1 and 2.

The other half of the experiment consisted of soft phosphate and ground limestone additions to a basal diet containing 2.5 percent fish meal (Table 7, diet 2). Supplemental levels of 0.30 and 0.40 percent phosphorus were fed with the supplemental calcium levels shown in the lower half of Table 10. Two positive control diets containing 2.5 percent fish meal, 0.70 percent total phosphorus with 0.71 percent and 0.91 percent total calcium, respectively, were also fed. These diets were composed of the basal shown in Table 7 (diet 2) and supplemental phosphorus and calcium added from dicalcium phosphate and ground limestone.

Results and Discussion

Experiment 1

A level of 0.50 percent supplemental phosphorus supplied from soft phosphate combined with 0.20 percent supplemental calcium produced the best growth rate of any treatment group; however, higher calcium levels significantly

depressed growth (Table 8). Body weights were significantly increased by the addition of 0.20 percent supplemental calcium to the diet containing 0.40 percent phosphorus supplied from soft phosphate. Increasing the supplemental phosphorus level from 0.50 to 0.60 percent, with optimal calcium levels, depressed growth. However, at the 0.60 percent phosphorus level it was necessary to feed a higher level of supplemental calcium in order to depress growth.

Growth at each level of phosphorus supplementation was significantly influenced by the calcium level of the diet. Even though total calcium and phosphorus levels were considerably above those normally felt to be adequate, no combination of calcium and phosphorus levels utilized resulted in body weights statistically equivalent to those of the positive control diets (Table 8).

Experiment 2

No level of supplemental phosphorus from soft phosphate, regardless of calcium level, supported body weights at four weeks of age that were equal to those obtained with the positive control diets (Table 9). All tibia ash values except those for the extreme lowest and highest calcium levels of the 0.40 percent supplemental phosphorus series were not significantly different from those for positive control diets. This effect is possibly

TABLE 8

Four-week body weights of chicks fed different levels of soft phosphate and ground limestone (experiment 1)

Supplemental[1] P (%)	Supplemental[1] Ca^2 (%)	Total Calcium (%)	4 wk. Body weight[5] (grams)
0.36[3]	0.16	0.80	479a
	0.36	1.00	491a
	. .	0.92	380g
	0.20	1.12	440cd
0.40[4]	0.30	1.22	432cd
	0.40	1.32	426de
	. .	1.12	450bc
	0.20	1.32	464b
0.50[4]	0.30	1.42	439cd
	0.40	1.52	430de
	. .	1.32	420ef
	0.20	1.52	439cd
0.60[4]	0.30	1.62	432de
	0.40	1.72	408f

[1]Basal diet contained 0.34 percent phosphorus and 0.16 percent calcium.

[2]Supplied as ground limestone.

[3]Supplied as dicalcium phosphate, and considered to be positive controls.

[4]Supplied as soft phosphate.

[5]Means with different letters are significantly different according to Duncan's multiple range test.

TABLE 9

Four-week body weights, feed conversion, and tibia ash of chicks fed different levels of soft phosphate and ground limestone (experiment 2)

Supplemental[1] P (%)	Ca[2] (%)	Total calcium	4 wk. Body weight[5] (grams)	Grams feed/ gram of body weight	Tibia ash[5] (%)
0.36[3]	0.16	0.80	510d	1.79	45.1bc
	0.36	1.00	517d	1.85	45.1bc
	. .	0.92	470bc	1.80	42.3a
	0.20	1.12	467bc	1.86	43.2ab
	0.25	1.17	463bc	1.82	43.8abc
0.40[4]	0.30	1.22	464bc	1.90	42.9ab
	0.35	1.27	446ab	1.86	43.9abc
	0.40	1.32	430a	1.94	41.6a
	. .	1.12	456abc	1.83	43.3ab
	0.10	1.22	476c	1.80	45.0bc
	0.20	1.32	452abc	1.89	45.6c
0.50[4]	0.25	1.37	468bc	1.84	45.6c
	0.30	1.42	470bc	1.90	44.8bc
	0.40	1.52	456abc	1.89	42.9ab
	. .	1.32	448abc	1.83	45.9c
	0.20	1.52	469bc	1.88	46.0c
0.60[4]	0.30	1.62	477c	1.87	44.9bc
	0.40	1.72	459bc	1.84	44.8bc

[1]Basal diet contained 0.34 percent phosphorus and 0.16 percent calcium.

[2]Supplied as ground limestone.

[3]Supplied as dicalcium phosphate, and considered to be positive controls.

[4]Supplied as soft phosphate.

[5]Means with different letters are significantly different according to Duncan's multiple range test.

explained by a changing calcium and phosphorus ratio. No statistically significant differences were found in feed efficiency values.

Experiment 3

No combination of supplemental phosphorus and calcium in diets without fish meal supported body weights at four or eight weeks of age that were equal to those produced with the positive control diets (Table 10). Highest four-week tibia ash values from diets without fish meal were obtained with 0.40 percent and 0.50 percent supplemental phosphorus from soft phosphate and 0.10 percent supplemental calcium. Both of these values were significantly greater than the bone ash of birds fed the positive control diet containing 0.70 percent total phosphorus and 0.80 percent total calcium. None of the tibia ash values from diets without fish meal were significantly different from those of birds fed the 0.70 percent phosphorus and 1.00 percent calcium positive control diet.

No combination of phosphorus and calcium supplementation supported maximum four-week body weights when the diet contained fish meal (Table 10). However, the weights of birds receiving fish meal diets were much closer to the controls than those of birds fed diets

TABLE 10

Four- and eight-week body weights, feed conversion, and tibia ash of chicks fed different levels of soft phosphate and ground limestone (experiment 3)

Supplemental P (%)	Supplemental Ca[1] (%)	Total Calcium (%)	Body weight (grams) 4 wk.[6]	Body weight (grams) 8 wk.[6]	Grams feed/gram of body weight 4 wk.[6]	Grams feed/gram of body weight 8 wk.	4 wk. Tibia ash[6] (%)
Without fish meal[2]							
0.36[3]	0.16	0.80	531ef	1519f	1.63abcd	2.08	42.0ab
	0.36	1.00	522e	1462def	1.64abcde	2.12	43.3abc
	. .	0.92	477ab	1353ab	1.59ab	2.12	43.1abc
	0.10	1.02	487abcd	1361abc	1.64abcde	2.09	45.1c
0.40[4]	0.20	1.12	490abcd	1341ab	1.57a	2.11	42.9abc
	0.30	1.22	464a	1321ab	1.62abc	2.11	41.8ab
	. .	1.12	486abcd	1384abcd	1.63abcd	2.11	44.4bc
0.50[4]	0.10	1.22	481abc	1364abc	1.65abcdef	2.16	45.2c
	0.20	1.32	468a	1328ab	1.70cdef	2.22	43.5abc

[1]Supplied as ground limestone.

[2]Basal diet contained 0.34 percent phosphorus and 0.16 percent calcium.

[3]Supplied as dicalcium phosphate, and considered to be positive controls.

[4]Supplied as soft phosphate.

[5]Basal diet contained 0.46 percent phosphorus and 0.28 percent calcium.

[6]Means with different letters are significantly different according to Duncan's multiple range test.

TABLE 10--*Continued*

Supplemental P (%)	Supplemental Ca[1] (%)	Total Calcium (%)	Body weight (grams) 4 wk.[6]	Body weight (grams) 8 wk.[6]	Grams feed/gram of body weight 4 wk.[6]	Grams feed/gram of body weight 8 wk.	4 wk. Tibia ash[6] (%)
With fish meal[5]							
0.24[3]	0.16	0.71	551f	1514f	1.67bcdef	2.14	43.6abc
	0.36	0.91	520e	1488ef	1.69cdef	2.22	43.6abc
	. .	0.89	508cde	1448cdef	1.74f	2.17	42.5abc
	0.10	0.99	509de	1405abcde	1.70cdef	2.18	43.5abc
0.30[4]	0.20	1.09	464a	1314a	1.73ef	2.28	41.2a
	0.30	1.19	503bcde	1411bcde	1.72def	2.19	43.3abc
	. .	1.08	510de	1452cdef	1.72def	2.13	43.6abc
0.40[4]	0.10	1.18	512de	1505f	1.66bcdef	2.11	44.1abc
	0.20	1.28	508cde	1461def	1.71cdef	2.19	43.8abc

[1]Supplied as ground limestone.

[2]Basal diet contained 0.34 percent phosphorus and 0.16 percent calcium.

[3]Supplied as dicalcium phosphate, and considered to be positive controls.

[4]Supplied as soft phosphate.

[5]Basal diet contained 0.46 percent phosphorus and 0.28 percent calcium.

[6]Means with different letters are significantly different according to Duncan's multiple range test.

containing no fish meal. Birds fed the fish meal diets containing 0.40 percent supplemental phosphorus from soft phosphate, regardless of the calcium level fed, had a body weight at eight weeks of age which was not statistically different from those of birds receiving the positive control diets (Table 10). The birds receiving fish meal and 0.30 percent added phosphorus with no supplemental calcium also had body weights at eight weeks of age that were not significantly different from the control groups. Four- and eight-week body weights of birds fed the diet with 0.30 percent supplemental phosphorus and 0.20 percent supplemental calcium were significantly lower than weights of birds fed most other diets containing fish meal. These weights are obviously not representative of the treatment since both higher and lower levels of supplemental calcium produced greater body weights. This variability was attributed to an unexplainable low weight for two of the replications within this treatment. None of the tibia ash values for the fish meal group were statistically different (Table 10).

Previous work (Waldroup *et al.*, 1965b) indicated that growth of chicks was greatly improved when a small amount of phosphate with a high availability was added to the diet. These data would indicate that the fish meal furnished some supplemental phosphorus of high availability,

and that this was necessary to obtain maximum growth of broiler-type chicks. The phosphorus in fish meal has been previously reported to be highly available (Waldroup *et al.*, 1965a).

Some significant differences were noted among feed efficiency values when four-week measurements were evaluated; however, these trends had disappeared by the time the birds reached eight weeks of age.

Summary

Three experiments were conducted to ascertain factors influencing the growth of broiler-type birds while utilizing soft phosphate as the sole source of supplemental phosphorus. A range of supplemental calcium levels was provided from ground limestone in order to eliminate the dietary calcium level as a limiting factor.

Four-week body weights equal to positive control diets could not be produced by diets containing soft phosphate as the sole supplemental phosphorus source. The addition of 2.5 percent fish meal to the basal diet improved four-week body weights; however, they still were not equal to the weights of birds receiving the positive control diet containing 0.80 percent calcium. The addition of 0.40 percent supplemental phosphorus and 0, 0.10, or

0.20 percent supplemental calcium to a diet containing 2.5 percent fish meal resulted in an eight-week average body weight statistically equal to the weight of the control groups. The improved response of the birds receiving fish meal diets was attributed to the supplemental phosphorus of high availability supplied by the fish meal.

CHAPTER 7

SUMMARY

At present, there are no standardized levels of calcium to be fed in an assay conducted to determine the biological value of feed grade phosphate materials. Some researchers feed a level of 1 percent calcium, regardless of the level of phosphorus in the diet; others utilize a constant calcium:phosphorus ratio over the entire range of phosphorus supplementation. The main thesis investigated and reported in this dissertation is that the calcium level necessary for expression of maximum biological availability by a phosphate source varies with the level of phosphorus supplied. Experiments were also conducted to investigate the influence of diet composition on the utilization of soft phosphate in broiler diets.

A series of 15 trials involving approximately 15,000 chicks was conducted in order to develop a more meaningful chick assay for determining the availability of phosphorus from various commercial phosphate sources. A diet primarily of all-plant origin, containing degerminated corn meal and soybean meal, was utilized in all assay studies. The diet was calculated to contain 22 percent protein, 0.30 percent phosphorus, 0.26 percent calcium, and 2,200 Calories of productive energy per kilogram.

The vitamin premix supplied 2,200 I.C.U. of vitamin D_3/kg. of diet in order to eliminate the possibility of this vitamin being a factor limiting chick performance. Two corn-soybean meal type basal diets, differing only in fish meal content, were used for the studies concerning diet composition.

All assay studies were of three weeks duration, starting when the chicks were randomly assigned to battery brooders at one day of age. Body weight and bone ash were used as the criteria of evaluation. On the twenty-first day of age all birds were weighed and representative bone samples taken for individual ash determinations. The studies of diet composition were of either four or eight weeks duration, starting when the chicks were housed in floor pens at one day of age. Body weight, bone ash, and feed conversion values were used for evaluation purposes.

Five supplemental phosphorus levels (0.05, 0.10, 0.15, 0.20, and 0.25 percent) were provided from reagent grade monosodium phosphate, which is the standard source that other phosphates are usually compared with in order to determine the biological value of the material tested. Three supplemental phosphorus levels (0.07, 0.14, and 0.21 percent) were furnished by each of the other sources tested--defluorinated phosphate, dicalcium phosphate, and soft phosphate. Five equally spaced supplemental calcium

levels, which varied with source and level of phosphorus, were provided from reagent grade calcium carbonate for each of the phosphorus additions.

All the data were evaluated statistically, and, for monosodium phosphate, levels of 0.44, 0.47, 0.50, 0.53, 0.56, and 0.59 percent total calcium were suggested for total phosphorus levels of 0.30, 0.35, 0.40, 0.45, 0.50, and 0.55 percent, respectively. A subsequent experiment indicated that these proposed calcium levels were adequate.

The total dietary calcium levels proposed as a standard curve for each of the remaining sources were as follows: 0.498, 0.590, and 0.685 percent for defluorinated phosphate; 0.47, 0.58, and 0.69 percent for dicalcium phosphate; and 0.56, 0.62, and 0.68 percent for soft phosphate with total phosphorus levels of 0.37, 0.44, and 0.51 percent, respectively. It should be emphasized that these curves are valid only when assay diets identical to those employed in the development of the curves are used.

Results of studies concerning the influence of diet composition on soft phosphate utilization in broiler diets indicated that the addition of 2.5 percent fish meal to a diet containing soft phosphate as the sole supplementary phosphorus source could produce body weights and bone ash equal to control groups supplemented with

dicalcium phosphate. Various dietary combinations of phosphorus supplied from soft phosphate and calcium supplied as calcium carbonate were fed in an attempt to produce growth and bone ash equal to that produced with commercial feeds. No combination of calcium and phosphorus levels alone produced the desired result; however, altering the diet composition with the inclusion of 2.5 percent fish meal in feeds containing 0.40 percent supplemental phosphorus and 0-0.20 percent supplemental calcium resulted in bone ash and body weight values not significantly different from those of controls. The response of the birds receiving fish meal diets was attributed to the highly available supplemental phosphorus supplied by the fish meal.

REFERENCES

Ammerman, C. B., H. W. Norton, and H. M. Scott, 1960. Rapid assay of inorganic phosphates for chicks. Poultry Sci. 39:245-250.

Association of Official Agricultural Chemists, 1965. Official Methods of Analysis, 10th Ed., Washington, D. C.

Couch, J. R., G. S. Fraps, and R. M. Sherwood, 1937. Vitamin D requirements of growing chicks as affected by the calcium content of the ration. Poultry Sci. 16:106-108.

Creech, B. G., B. L. Reid, and J. R. Couch, 1956. Evaluation of dicalcium phosphate supplement as a source of phosphorus for chicks. 1. Comparison of dicalcium and tricalcium phosphate as a source of phosphorus in chick and poult rations. Poultry Sci. 35:654-658.

Duncan, D. B., 1955. Multiple range and multiple F tests. Biometrics, 11:1-42.

Ewing, W. R., 1963. Poultry Nutrition, 5th Ed. The Ray Ewing Company, Pasadena, California.

Fisher, H., E. P. Singsen, and L. D. Matterson, 1953. The influence of feed efficiency on the phosphorus requirement for growth and bone calcification in the chick. Poultry Sci. 32:749-754.

Fritz, J. C., and T. Roberts, 1966. Influence of levels of vitamin D and calcium on the utilization of phosphorus by the growing chick. Poultry Sci. 45:1085-1086.

Gardiner, E. E., H. E. Parker, and C. W. Carrick, 1959. Soft phosphate in chick rations. Poultry Sci. 38:721-727.

Gillis, M. B., L. C. Norris, and G. F. Heuser, 1949. The effect of phytin on the phosphorus requirement of the chick. Poultry Sci. 28:283-288.

Gillis, M. B., L. C. Norris, and G. F. Heuser, 1954. Studies on the biological value of inorganic phosphates. J. Nutrition 52:115-125.

Grau, C. R., and P. A. Zweigart, 1953. Phosphatic clay as a phosphorus source for chicks. Poultry Sci. 32:500-503.

McGinnis, J., L. C. Norris, and G. F. Heuser, 1944. Poor utilization of phosphorus in cereals and legumes by chicks for bone development. Poultry Sci. 23:157-159.

McKnight, W. F., and A. B. Watts, 1966. The effect of vitamin D_3 on the utilization of phosphorus from various sources. Poultry Sci. 45:1104.

Maddy, K. H., R. B. Grainger, W. A. Dudley, and F. Puchal, 1963. The application of linear programmming to feed formulation. Feedstuffs 35 (5):28-30.

Matterson, L. D., E. P. Singsen, and H. M. Scott, 1945. Rock phosphates as phosphorus supplements for the growing chick. Poultry Sci. 24:188-190.

Motzok, I., D. Arthur, and H. D. Branion, 1965. Factors affecting the utilization of calcium and phosphorus from soft phosphate by chicks. Poultry Sci. 44:1261-1270.

National Research Council, 1966. Nutrient requirements of domestic animals. Nutrient requirements for poultry. Washington D. C.

Nelson, T. S., and H. T. Peeler, 1961. The availability of phosphorus from single and combined phosphates to chicks. Poultry Sci. 40:1321-1328.

Nelson, T. S., and H. T. Peeler, 1964. Current status of biological testing of feed phosphates. Feedstuffs 36 (11):32.

Nelson, T. S., and H. C. Walker, 1964. The biological evaluation of phosphorus compounds. A summary. Poultry Sci. 43:94-98.

O'Rourke, W. R., P. H. Phillips, and W. W. Cravens, 1952. The phosphorus requirements of growing chickens as related to age. Poultry Sci. 31:962-966.

Singsen, E. P., L. D. Matterson, and H. M. Scott, 1947. Phosphorus in poultry nutrition. III. The relationship between the source of vitamin D and the utilization of cereal phosphorus by poults. J. Nutrition 33:13-26.

Singsen, E. P., and H. M. Scott, 1946. Phosphorus in poultry nutrition. II. Sodium acid phosphate, tri-calcium phosphate and bonemeal as sources of phosphorus for the growing chick. Poultry Sci. 25:302-303.

Singsen, E. P., H. M. Scott, and L. D. Matterson, 1948. The phosphorus requirement of the chick. Storrs Agr. Exp. Sta. Bull. 260.

Snedecor, G. W., 1956. Statistical Methods, 5th Ed. The Iowa State College Press, Ames, Iowa.

Summers, J. D., S. J. Slinger, W. F. Pepper, I. Motzok, and G. C. Ashton, 1959. Availability of phosphorus in soft phosphate and phosphoric acid and the effect of acidulation of soft phosphate. Poultry Sci. 38:1168-1179.

Vandepopuliere, J. M., C. B. Ammerman, and R. H. Harms, 1961. The relationship of calcium:phosphorus ratios to the utilization of plant and inorganic phosphorus by the chick. Poultry Sci. 40:951-957.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms, 1963. Calcium and phosphorus requirements of finishing broilers using phosphorus sources of low and high availability. Poultry Sci. 42:752-757.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms, 1965a. A comparison of phosphorus assay techniques with chicks. Poultry Sci. 44:1086-1089.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms, 1965b. The utilization of phosphorus from animal protein sources for chicks. Poultry Sci. 44:1302-1306.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms, 1965c. Studies on the acidulation of soft phosphate. Poultry Sci. 44:1519-1523.

BIOGRAPHICAL SKETCH

Bobby Leon Damron was born November 6, 1941, at Ocala, Florida. He was graduated from Gainesville High School in June, 1959. In May, 1963, he received the degree of Bachelor of Science in Agriculture with high honors from the University of Florida. He also completed the requirements for teacher certification in Vocational Agriculture in May, 1963. He worked as an undergraduate assistant in the Department of Poultry Science from September, 1961 to May, 1963.

In September, 1963, he became a Research Assistant in the Poultry Science Department and received the Master of Science in Agriculture degree in December, 1964.

In January, 1966, he accepted the position of Interim Research Associate and Instructor and continues in that capacity at the present time.

The author is a member of the Poultry Science Association, Alpha Zeta, Gamma Sigma Delta, Alpha Tau Alpha, the Poultry Science Club, a past Vice-President of the Collegiate F.F.A. Chapter, and a past President of the Collegiate 4-H Club.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

March, 1968

Dean, College of Agriculture

Dean, Graduate School

Supervisory Committee:

R H Harms

Chairman

J S Moore

H R Wilson

C.B. Ammerman